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Structure of the PTEN Tumor Suppressor Associated with the Fluid Lipid Membranes

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e tumor suppressor PTEN[1] is a phosphatase involved in the regulation of PI (3,4,5)P_3. It consists of a phosphatase and a C2 domain that interact synergistically with anionic lipids in membranes. A single-point mutation of the wild type (wt) protein, H93R, has the same secondary structure but significantly reduced enzyme activity. As for many membrane proteins, the crystal structure of (a truncated) PTEN has been determined[2], but the association of the protein with lipid membranes has only been indirectly inferred. We study the association of wt PTEN and H93R with membranes using neutron reflectometry (NR) of tethered bilayer lipid membranes (tBLMs)[3,4] which are long-term stable and retain their fluidity with in-plane dynamics similar to that in vesicles.[5] Surface Plasmon Resonance (SPR) spectroscopy has also been used to investigate the binding of these two variants of PTEN.

Data analysis uses a composition-space model that resolves the thermally disordered membrane with Ångstrom resolution,[6] enabling us to characterize PTEN association with the bilayer in its physiologically relevant, disordered state. The crystal structure serves as a starting point for model refinement. Computational techniques are used to explore the conformational flexibility of the peptide stretches deleted for crystallization. We observed slight differences in the peripheral association of H93R and wt PTEN with the bilayer's headgroup region and speculate how these may be related to their functional distinctions.

(1) I. Sansal *et al*., 2004, *J. Clin. Oncology* 22:2954.
(2) J. Lee,*et al*., 1999, *Cell* 99:323.
(3) D. J. McGillivray *et al*., 2007, *Biointerphases* 2:21.
(4) F. Heinrich *et al*., 2009, *Langmuir* 25:4219.
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(6) F. Heinrich *et al*., *J. Appl. Phys.*, submitted.

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An Improved Open Microfluidic Flow Cell for Measuring Solute Adsorption to Monolayers

Howard L. Brockman, Dmitry Malakhov, William E. Momsen, Maureen M. Momsen.

We earlier described an apparatus and optical method that permit quantitative measurement of the adsorption of a solute to a gas-liquid interface [K.C. Hoang, D. Malakhov, W.E. Momsen and H.L. Brockman (2006) Anal. Chem. 78, 1657-1664]. The interface may or may not support a protein, lipid or other monolayer. A potential limitation to such a flow cell, being probed optically through the monolayer, is concomitant solute adsorption to the solid trough bottom, which is parallel to but displaced from the monolayer being studied. To circumvent this unwanted adsorption we introduced a flowing sucrose solution under the flowing solute solution that supports the monolayer. In this way solute is prevented from reaching the trough bottom and any solute which might adsorb to or diffuse through the sucrose-solute solution interface is rapidly moved from the optical path by flow to the trough drain. Eliminating the need to chemically modify the trough bottom by introducing a sucrose layer allows trough dimensions to be reduced, allows robust and fully automated cleaning between experiments and improves reproducibility of solute adsorption measurements. An additional feature of the new configuration is control of the thickness of the solute solution layer, which conserves reagents and enhances the sensitivity of solute adsorption measurements. The apparatus was used to characterize the interaction of a fluorescent protein antigen with an antibody monolayer and to the initial rate of adsorption of bovine serum albumin to phospholipid-diacylglycerol monolayers of differing lipid compositions but approximately constant surface pressure.

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Achieving Ultralow Fouling Performance for Poly(Hydroxy-Functional Methacrylates) Grafted Surfaces via Atom Transfer Radical Polymerization

Chao Zhao, Qiuming Wang, Jun Zhao, Xiang Yu.

The development of nonfouling biomaterials to prevent nonspecific protein adsorption and cell/bacterial adhesion is critical for many biomedical applications, such as antithrombogenic implants and biosensors. In this work, we synthesize and characterize two polymer brushes on the gold substrate by using surface-initiated atom transfer radical polymerization (SI-ATRP) of Hydroxy-Functional Methacrylates monomers: HEMA and HPMA. We investigate the effect of film thickness on protein adsorption from single protein solution to complex human blood media, as well as bacterial adhesion. Surface plasmon resonance (SPR) results show a correlation between antifouling properties and film thickness, that is, optimal film thickness of 25-45 nm for polyHPMA and 20-45 nm for polyHEMA is obtained to achieve almost zero protein adsorption (< 0.3 ng/cm^2) from single protein solution, 10% human blood plasma, and 10% human blood serum. Furthermore, polyHEMA brushes remain its excellent resistance to 100% human serum and plasma with < 5 ng/cm^2 adsorption amount, while polyHPMA brushes adsorb more proteins of 13.5 ng/cm^2 and 50.0 ng/cm^2 from 100% human serum and plasma, respectively. More strikingly, static bacteria adhesion assay shows that there are almost no bacteria adhered to polyHEMA and polyHPMA surfaces as compared to full coverage of bacteria on the bare gold surface. In addition, stability tests show a very stable polyHPMA and polyHEMA surfaces without polymer degradation within 20 days. PolyHEMA and PolyHPMA provide effective nonfouling biomaterials, alternative to poly (ethylene glycol), to highly resist nonspecific protein adsorption and cell/bacteria adhesion.

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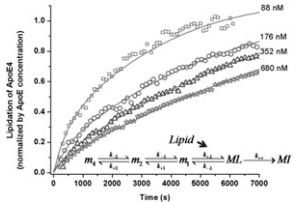
Association-Dissociation Behavior of the Apolipoprotein E Proteins: Implications for Lipid Binding

Kanchan Garai, Carl Frieden.

Apolipoprotein-E4 (ApoE4) is a risk factor for Alzheimer's diseases but the structural or functional differences between the isoforms viz, ApoE2, ApoE3 and ApoE4 are unknown. Lipidation of ApoE is important for its functions. However, the molecular mechanism and the isoform specificity of ApoE-lipid interactions are unclear. In vitro lipid free ApoE undergoes self association but which oligomeric form of ApoE interacts with lipids is unknown. Using Fluorescence Correlation Spectroscopy, intermolecular FRET and sedimentation methods we find that association-dissociation reaction of ApoE can be modeled by a monomer-dimer-tetramer process. Dissociation kinetics as measured by changes in FRET show two phases reflecting dissociation of tetramer to dimer and of dimer to monomer. The rate constants are found to be different for the ApoE isoforms. The kinetics of lipidation of ApoE in presence of unilamellar vesicles of DMPC show striking similarity with the kinetics of dissociation of ApoE multimers to monomers. Furthermore, lipidation kinetics are slower at higher ApoE concentrations and kinetic data are consistent with only monomers binding to lipids. The results imply that differences in lipidation properties between apoE isoforms arise due to their differences in association-dissociation behavior.



2775-Pos Board B761

Ligand Induced Conformational Redistribution in Synaptotagmin I C2A

Jacob W. Gauer, Samantha R. Jaworski, R. Bryan Sutton, Greg Gillispie, Anne Hinderliter.

Thermodynamic parameters capture the overall contribution to a system's energetics. In the case of binding proteins, such as Synaptotagmin I, ascertaining the overall magnitude of the interactions within the protein is the first step toward addressing how energy is distributed throughout the body of the protein. Our aim is to understand how the signal of ligand binding is disseminated through the protein during the role it plays in regulated exocytosis. While several detailed molecular approaches have identified putative regions where interactions occur, it is the energetics that are key to understanding Synaptotagmin I's functional response. Here, denaturation studies of the C2A domain of Synaptotagmin I were carried out in conditions that are physiologically relevant to regulated exocytosis where calcium ions and phospholipids were either present or absent. Denaturation was carried out using two techniques: differential scanning calorimetry (DSC) and fluorescence lifetime (FLT). A global analysis approach combining these data sets was used where the data was simultaneously fit to models derived from thermodynamic principles. The enthalpy associated with the denaturation of the C2A domain of Synaptotagmin I in the absence of all ligands was found to be quite low when compared to other proteins of the similar molecular weight, which suggests that the protein exhibits conformational flexibility. In addition, the denaturation behavior is shown to change

when ligand is bound, which suggest a similar change in the conformational flexibility.

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Unraveling the Specific Interactions of Collectin with Lipopolysaccharides

Samuel Gourion-Arsiquaud, Barbara A. Seaton, James F. Heads, Francis X. McCormack, Carol R. Flach, Richard Mendelsohn.

Our laboratory is investigating how protein and lipid structures are modified during their mutual interaction and how these specific interactions are linked to important physiological functions, especially at the level of pulmonary surfactant. Surfactant protein A (SP-A), one of the main pulmonary collectin, is involved in several fundamental biological functions including surfactant homeostasis, biophysical activity, and host defense. The current study focuses on the molecular basis of collectin/lipopolysaccharide (LPS) recognition in samples relevant to host-defense especially those related to SP-A.

SP-A binds to macrophages in the lung and induces chemotaxis and phagocytosis of a variety of pathogenic microbial species. Although the general mechanism by which SP-A functions is known, its interaction with the outer membrane of Gram-negative bacteria at the molecular level remains elusive. SP-A is suggested to bind to Lipid A of bacteria through lipopolysaccharide, to promote aggregation and to destabilize bacterial membranes. To unravel the molecular basis of this mechanism, the bulk-phase interaction between SP-A and Lipid A was investigated by FTIR spectroscopy. This powerful structure/function investigation tool, provides quantitative information about structures with direct biological relevance such as the conformation of the secondary structure of the protein as well as lipid acyl chain conformation, orientation and polar head group interaction in samples. This is particularly interesting when the traditional high resolution approaches such as X-ray diffraction cannot provide valuable insights.

To determine the nature of the recognition between Lipid A and SP-A and localize the specific domains involved, we used wild-type and relevant SP-A mutants to monitor collectin binding perturbations in the acyl chain conformational order in the Lipid A as a function of temperature. The possible effects of ligand binding on protein backbone structure were predicted and tested through the simulations of the IR Amide I contour.

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Membrane Affinity and Neurotoxicity of β-Amyloid Oligomers

Rima Budvytyte.

A central event in pathogenesis of Alzheimer's disease (AD) is thought to be intracellular and extracellular accumulation of low molecular mass peptides - β-amyloids (Aβ). Soluble oligomers are the most cytotoxic form of Aβ although it is still unclear which size and morphology of the aggregates exert neurotoxicity [1]. In this work different sizes of soluble synthetic Aβ oligomers were used to determine their affinity to artificial phospholipid membranes as well as their toxicity to rat cerebellar granule cells (CGC) [2]. Oligomer binding strength was estimated by the fluorescence correlation spectroscopy (FCS). Membrane composition was found to be one of the important factors affecting binding of the Aβ oligomers to phospholipid vesicles. Both small Aβ oligomers (with apparent heights of 1-2 nm, as measured by AFM) and bigger aggregates (heights above 4-5 nm) exhibit binding affinities to phospholipid vesicles in the sub-micromolar range. However, differently sized Aβ oligomers exhibited different levels of neurotoxicity in CGC toxicity tests. Small oligomers induced rapid neuronal necrosis in sub-micromolar concentrations while the bigger ones did not. Instead, rather than inducing imediate neuronal death, Aβ aggregates decreased density of CGC in the culture dish suggesting apoptotic elimination of cells affected by the amyloid species.

1.Charles G. Glabe. *J. Biol. Chem.*,2008, 283:29639.

2.Paulius Cizas, Rima Budvytyte *et al.*, *Arch. Biochem. Biophys.*, 2010, 496:84.

2778-Pos Board B764

In situ Measurement of Human islet Amyloid Polypeptide Misfolding at Lipid/Water Interfaces Probed by sum Frequency Generation Spectroscopy

Li Fu, Gang Ma, Elsa Yan.

Kinetic analysis of conformational changes of proteins at interfaces is crucial for understanding many biological processes at membrane surfaces. We applied surface-selective sum frequency generation (SFG) spectroscopy to investigate the kinetics of conformational changes of an intrinsically disordered protein, human islet amyloid polypeptide (hIAPP), which is known to misfold into β-sheet upon interaction with the lipid/water interface. We observed changes in the amide I spectra of hIAPP in the presence of dipalmitoylphosphoglycerol (DPPG) that correspond to lipid-induced changes in protein secondary structures. We obtained both the achiral and chiral SFG spectra. Using the achiral-sensitive ssp (s-polarized SFG, s-polarized 800 nm, p-polarized infrared) polarization, we observed a gradual shift of the amide I spectrum of hIAPP. Using the chiral-sensitive psp polarization, we observed a gradual buildup of the chiral structures that display characteristics of parallel β-sheets, including a dominant peak at ~1620 cm^{-1} and a shoulder at 1660 cm^{-1}, which is in good agreement with the theoretical prediction based on the hIAPP parallel β-sheet structure. Moreover, we used ab inito calculation to obtain the hyperpolarizibility of the hIAPP β-sheet aggregates, from which we determined the orientational angle of the β-sheet strand to be ~45° at interfaces. We discuss the implication of such insertion of the β-sheet aggregates into the membrane in relation to the hIAPP induced permeability in cell membrane. Also, we propose that the second-order chiral-optical response from the hIAPP β-sheet aggregates could be a highly characteristic optical property for other amyloid, and thus potentially useful for in situ amyloid detection. Our study demonstrates that SFG is an in situ and label-free technique, which can provide both kinetic and structural information to probe protein conformational changes at interfaces.

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Interaction of Monomeric Amyloid Beta-Protein (ABETA-42)with Bilayers Containing Anionic Phospholipids

Hasna Ahyayauch, Michal Raab, Alicia Alonso, Igor Tvaroska, Massimo Masserini, Felix M Goni.

Alzheimer's disease (AD) is a late-onset neurological disorder with progressive loss of memory and cognitive abilities as a result of excessive neurodegeneration. AD is characterized by extracellular aggregates of β-amyloid (Aβ) peptides known as amyloid plaques. The toxicity of the peptide appears to require conversion of the monomeric form to an aggregated fibrillar species. Electrostatic interactions between Aβ and the phospholipid headgroup have been found to control the association and insertion of monomeric Aβ into membrane. To elucidate the molecular and structural details of Aβ-membrane association, we have used LUV and monolayers composed of sphingomyelin (SM), cholesterol (Ch) ± dimyristoyl phosphatidic acid (DMPA). Isothermal titration calorimetry (ITC), Langmuir monolayer experiments and molecular dynamics (MD) simulations have been performed. Using Langmuir monolayers and ITC, our results indicate that Aβ(42) inserts in bilayers formed by SM/Ch/DMPA (40/40/20) and SM/Ch/DMPA (47.5/47.5/5), but less so in those consisting of SM/Ch in the absence of DMPA. The effect obtained by DMPA (5%) is more important than that of DMPA (20%). Moreover, the molecular dynamics simulations of Aβ42 on the surface of the phospholipid bilayer show different binding modes in models with varying DMPA ratios. The 5% DMPA surface binds to Aβ42 more strongly than the one containing 20% DMPA and conformational changes occur in the secondary structure. The 20% DMPA surface binds weakly, and the native secondary structure of Aβ42 is conserved.

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Membrane Interactions of Antimicrobial Retro-Lactoferricin Peptides by Solid-State NMR and Partitioning Assays

Amir V. Francis, Denise V. Greathouse.

Bovine lactoferricin (LfB25) is a 25-residue peptide released from the N-terminal of bovine lactoferrin which exhibits broad spectrum antimicrobial properties. A hexapeptide, LfB6 (**RRWQWR-NH_2**), is a small fragment that retains significant antimicrobial activity (Tomita et al. 1994. *Acta Paediatr Jpn.* 36:585). We seek to modify and understand the antimicrobial properties of LfB6 through various modifications. Previous studies from our lab have shown that antimicrobial activity can be increased by N-acylation and Trp-methylation (Greathouse et al. 2008. *J. Pept. Sci.* 14:1103), as well as by reversal of the peptide sequence (Retro-LfB6). Acylation and Trp-methylation enhance the binding of the peptides to bacterial-like membranes. In addition, Trp-methylation allows for selective ring labeling with deuterium for solid-state NMR studies. To determine whether combining the three modifications (sequence reversal, Trp methylation, and acylation) will further enhance activity, two Retro-LfB6 peptides have been synthesized with a methylated Trp at sequence position 4. One of the peptides has been further modified by N-acylation with a 6-carbon fatty acid. Retro-LfB6 **(RWQMeWRR-NH_2)**